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Multivariate assessment of azo dyes' biological activity parameters

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ABSTRACT

Lipophilicity as key molecular descriptor of potential biological activity for selected derivatives of azo dyes was determined mathematically, by using relevant software packages and by reversed-phase thin-layer chromatography (RPTLC) on C18 and cyano modified carriers in mixtures of water/*n*-propanol and water/acetone. The obtained chromatographic parameters, R_M^0 and *m*, of the examined azo dyes were correlated with the standard measure of lipophilicity, log *P*, important pharmacokinetic predictors and selected toxicity parameters applying linear regression analysis. Thereby, good correlations for each applied system were obtained (average correlation coefficient, r, 0.944, 0.885 and 0.919). Also, the correlations between the studied parameters of azo dyes were examined applying two multivariate methods (Cluster Analysis and Principal Component Analysis). It was shown that the polarity of the substituent, and to a lesser extent its electronic effects has the greatest influence on the studied parameters of the azo dyes derivatives. Multivariate methods pointed out the similarity of the chromatographic retention constant, R_M^0, with the parameters of lipophilicity, unlike the chromatographic parameter *m*, which exhibits better agreement with the toxicity parameters.

1. Introduction

The human need for various, stable and easily applicable dyes has resulted in the production of about 10,000 tons of different coloring agents annually [1]. Among them, azo dyes represent the most diverse and the most widely used group of synthetic dyes in industry [2–5]. In comparison to other synthetic dyes, they are characterized by intensity, brightness, wide diapason of colors, stability, high moisture resistance and practical handling. However, due to poor utilization, even 20–40% of the initial dye does not react and finally ends up in the wastewater [6,7]. Also, azo dyes and their products, mainly aromatic amines, are large environmental pollutants because of their hard degradability, toxicity [8,9], carcinogenicity [10,11] and mutagenicity [12]. Therefore, it is necessary to harmonize their qualitative properties, the requirements of the society and the ecological regulations for the possibility of using newly synthesized azo dyes [13–18]. The answer on these requests can be given by mathematical models (QSAR, QSPR and QSRR) that enable the establishment of quantitative dependencies between structure, physical-chemical properties and activity of molecules [19–22]. The first step toward this goal is the selection of relevant molecular descriptors, among which lipophilicity plays a crucial role as an indicator of the potential biological activity of the compound [23–26]. It can be determined in different ways, and it is commonly expressed by partition coefficient, log *P* [27]. In recent studies, chromatographic parameters R_M^0 and *m*, obtained by reverse phase thin layer chromatography, are increasingly used to describe lipophilicity [28–34]. The reason for this is method's simplicity, applicability, efficiency and cost effectiveness.

The comprehensive studies of the compounds' biological potential beside lipophilicity include information on its bioavailability, pharmacokinetic properties and toxicity. The rate and extent to which the bioactive substance is absorbed and becomes available to the target site of the action represents the bioavailability. The level of intestinal absorption could be described by pharmacokinetic parameter Human effective permeability in jejunum, P_{eff} [35]. Molecules with higher lipophilicity more easily pass through the phospholipid bilayer of enterocytes and they are characterized by higher P_{eff} values [36]. By crossing into blood, the molecule can bind to the plasma proteins and its efficiency is described by pharmacokinetic parameter Plasma protein binding, *PPB* [37]. The possible passage of compound from blood into central nervous system is given by the value of pharmacokinetic parameter *BBB* (distribution through the blood-brain barrier, *BBB*) [38]. The *BBB* values higher than 0.4. shows that the compound has good predispositions as neurologically active substances, while the values of the *BBB* lesser than - 1 signify the impossibility of passing [39].

Bearing in the mind that the azo dyes has the first toxicity effects in the aquatic ecosystems, preliminary examinations have been performed on the organisms such as Algae, Daphnia, Medaka and Minnow [40].

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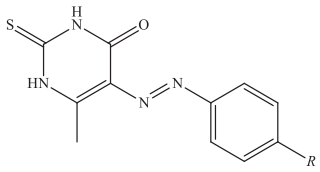
Table 1
The structure of the tested azo dyes.

R
H
Cl
Br
F
NO_2
OH
COOH
$COCH_3$
CH_3
OCH_3



In this study, the selected parameters of the biological activity of the azo dyes were obtained experimentally (thin layer chromatography on different mobile and stationary phases) and mathematically. All of them were analyzed by using different hemometric methods (linear regression, Cluster analysis, Principal component analysis). Based on the obtained results, the qualitative and/or quantitative dependence of the parameters of the biological activity of the azo derivatives in a function of the chemical structure of the analyzed compounds, as well as their relationships are defined.

2. Material and methods

The structures of the investigated azo dyes are presented in Table 1 and their synthesis and characterization are described in the literature [41].

In a QSAR analysis, a key molecular descriptor that can indicate the potential biological activity of a compound is lipophilicity. For experimental determination of lipophilicity, thin-layer chromatography on reverse C- 18 phases is traditionally applied, although more and more publications have been devoted to the use of other modified carriers of the stationary phase [42,43]. Inspired by this idea, in this paper, lipophilicity of the selected azo dyes was first determined experimentally by thin layer chromatography.

As carriers of the stationary phase, HPTLC RP-18W/UV_{254} (Macherey-Nagel GmBH, Germany) and Silica gel 60 CN F254s (Merck, Darmstadt, Germany) were used. The development of chromatograms was performed at room temperature with one-dimensional input technique without prior saturation of the chromatographic chamber atmosphere with solvent vapor. About 0.2 µl of the solution of the investigated azo dyes was applied to the chromatographic plates and the development of the chromatogram was carried out in the following solvent systems: water – *n*-propanol ($\varphi_{(n\text{-propanol})}$ = 0.32.–0 48.) and water – acetone ($\varphi_{(acetone)}$ = 0.32.–0 48).

The volume fraction of the organic solvent, φ, was varied in the amount of 0.04. The required time for developing the chromatogram was 20 min, and the front of solvent was approximately 5 cm. The identification of tested compounds was performed under UV light wavelength λ = 254 nm.

For each tested compound, the R_M value was calculated. The dependence of the calculated R_M values on the content of the organic modifier, φ, as the intercept, gives the chromatographic retention constant R_M^0, and the slope is the value of m [44]. Lipophilicity of the examined azo dyes was determined mathematically, also. The values of partition coefficient, log *P*, for investigated compounds were calculated using the VCCLAB software package [45]. Due to the wide use of azo dyes in different industries, it is also important to know their pharmacokinetic properties. For this purpose, values of important pharmacokinetic predictors - human effective permeability in jejunum (P_{eff}), plasma protein binding (*PPB*), and distribution through the blood brain barrier (*BBB*) were calculated using the Simulation Plus software [46].

For newly synthesized compounds it is inevitable to examine whether they have a potentially toxic effect on the ecosystem. With that goal, for the investigated azo dyes using the PreADMET computer software, the value of the effective concentration (EC_{50}) of the acute toxicity for a specific test organisms (Algae, Daphnia, Medaka, Minnow) were calculated [47].

The Origin 6.1 software was used for the processing of obtained experimental results. Hemometric calculations were made using the Statistics 13 software (StatSoft Inc., Tulsa, OK, USA).

Table 2
The chromatographic parameters R_M^0, *m*, r obtained for the tested azo dyes on reversed C- 18 phase in the both applied modifiers.

R	Modifier					
	n-Propanol			Acetone		
	R_M^0	*m*	r	R_M^0	*m*	r
H	0.411	−1.970	0.999	0.515	−1.312	0.998
Cl	0.540	−2.432	0.998	0.625	−1.531	0.996
Br	0.603	−2.555	0.998	0.720	−1.636	0.996
F	0.472	−2.202	0.996	0.549	−1.360	0.997
NO_2	0.136	−1.338	0.998	0.173	−0.738	0.995
OH	0.337	−1.705	0.998	0.425	−1.116	0.999
COOH	0.055	−0.931	0.999	0.096	−0.605	0.999
$COCH_3$	0.075	−1.062	0.998	0.115	−0.675	0.998
CH_3	0.557	−2.451	0.998	0.640	−1.570	0.998
OCH_3	0.385	−1.940	0.999	0.489	−1.209	0.998

3. Results and discussion

Determination of lipophilicity of the selected azo dyes using thin layer chromatography on different stationary phase

The obtained chromatographic parameters of the tested compounds are presented in Table 2 and Table 3 for C- 18 and CN carriers of the stationary phases, respectively.

From Table 2 and Table 3 it is obvious that the values of the obtained chromatographic retention parameters of the tested azo dyes depend on the several factors. These factors are: the nature of the used organic modifier, the applied stationary phase carriers and the nature of the substituent present in the molecule.

Comparing the values of the chromatographic parameters obtained in both used stationary phases, it can be noted that slightly higher values of the chromatographic parameter R_M^0 are obtained in the aprotic acetone. Because the chromatographic parameter R_M^0 depends solely on the nature of the substance and not from the used solvent, it was expected that the values would be approximately equal. However, the observed deviation is a common case in experimental work [48].

If the values of both chromatographic parameters R_M^0 and *m* obtained in different stationary phases are compared, then the values of

Table 3
The chromatographic parameters R_M^0, *m*, r obtained for the tested azo dyes on reversed CN phase in the both applied modifiers.

R	Modifier					
	n-Propanol			Acetone		
	R_M^0	*m*	r	R_M^0	*m*	r
H	0.670	−2.922	0.997	0.756	−2.785	0.994
Cl	0.775	−3.030	0.999	0.845	−2.828	0.998
Br	0.855	−3.090	0.999	0.917	−2.900	0.999
F	0.713	−2.969	0.997	0.789	−2.808	0.995
NO_2	0.327	−2.608	0.997	0.364	−2.530	0.999
OH	0.601	−2.838	0.998	0.674	−2.726	0.996
COOH	0.152	−2.482	0.998	0.202	−2.420	0.999
$COCH_3$	0.222	−2.548	0.997	0.266	−2.480	0.998
CH_3	0.795	−3.048	0.999	0.866	−2.885	0.999
OCH_3	0.628	−2.868	0.998	0.706	−2.743	0.997